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Sections and Section Cutting.

WITH A DESCRIPTION OF A NEW POLY-MICROTOME FOR FREEZING.

By WILLIAM HAILES, M. D.,

Assistant Physician, New York State Lunatic Asylum, Professor of Histology and Pathological Anatomy Albany Medical College, etc., etc., etc.

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SECTIONS AND SECTION CUTTING—WITH A DESCRIPTION OF A NEW POLY-MICROTOME FOR FREEZING.

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We are too often, as professional men, inclined to undervalue the importance of manipulative skill and mechanical detail in the preparation of objects intended for microscopic observation. One must not forget how entirely dependent we occasionally are, upon mechanical means to carry forward plans of investigation to a successful termination; as, for example, in the cutting of an embryo, of only a millimetre or so in length, into a series of one hundred or more successive sections, without losing one. An unbroken series is of great importance especially when studying the formation of organs. If we have the proper mechanical means at disposal, our labors are wonderfully lightened, and the preparations are in far better condition for that close scrutiny which must necessarily follow, and which is becoming more and more essential as we are called upon to deal with problems more intricate and abstruse in their nature, and demanding greater care and accuracy in methods of preparation.

There can be no question of the great importance of the examination of fresh tissues, and the desirability of being able to make free-hand sections of fresh organs for immediate microscopic examination. It is a matter not always sufficiently insisted upon, and is one of the very first, and also the very last thing required by

Prof. Von Recklinghausen and others in the pathological laboratories of the Old World.

We all know how difficult it is for beginners to make sections of fresh tissues sufficiently thin for satisfactory minute examination, and it is surprising, at the same time, to find how much can be readily accomplished by the aid of a little practice and a good razor; but all do not possess the requisite mechanical dexterity, nor sufficient of that cardinal virtue, patience, and must, therefore, resort to other means. My attention was particularly drawn to this point while at the University of Edinburgh, Scotland, in September last, by my friend, David J. Hamilton, F. R. C. S., etc., who is in charge of the pathological laboratories there, and to whom I am indebted for many valuable suggestions in relation to freezing, etc., etc. He has written a very interesting article in the *Journal of Anatomy and Physiology*, Vol. XII, entitled, "A New Method of Preparing Large Sections of Nervous Centers for Microscopic Investigation." He says, "it was found that the crystals of ice so broke up the delicate nervous tissue as to render it totally useless for minute examination," and discovered that "when brain substance was soaked in strong syrup, previous to freezing, it could be cut without the slightest injury. The sugar somewhat retards the freezing, and seems, besides, to alter the manner of crystallization, so that, instead of the ice being spicular in form, it, evidently, becomes granular, and does no injury to the parts." The specimen being placed in the solution, (sugar two ounces, water one ounce), for twenty-four hours, is removed to ordinary mucilage acaciæ for forty-eight hours, and is then cut in the freezing microtome.

Some years since it occurred to me that it was possible to construct a microtome combining several sizes in

one, and, after experimenting, I devised the following instrument, as indicated by the accompanying drawing, and which I would name

A POLY—MICROTOME FOR FREEZING.

ARRANGED FOR FREEZING WITH ICE AND SALT.

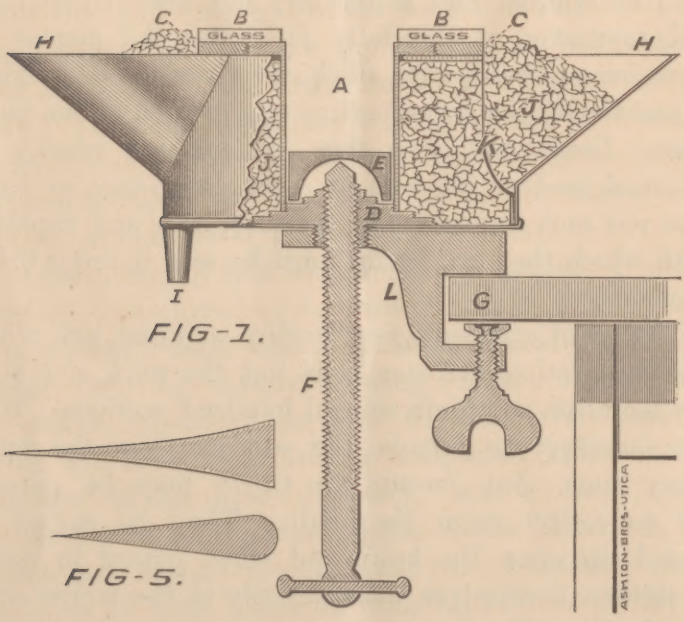


FIG-1.

FIG-5.

EXPLANATION OF FIG. 1.

(Drawing—One-fourth the original size).

A—Medium-sized cylinder. B—Plate-glass top, (to facilitate cutting). C—Metal face-plate. D—Pyramidal bed-plate, (containing five sizes). E—Plunger of microtome. F—Micrometer screw. L—Clamp. G—Table. H—Hoppers for feeding ice and salt. I—Exit tube for water. J—Ice and salt. K—Movable cover fitting inside of jacket to prevent the ice from escaping over the top. The outside of the ice-jacket is covered with felt or gutta percha.

EXPLANATION OF FIG. 5.

Sections of two knives, (5 in. long by 1¼ in. wide), and (4 in. by 1 in.)—recommended by Bevan Lewis, F. R. M. S.

One can convert this instrument into a larger or smaller microtome by simply selecting the desired sized

cylinder and plunger, and screwing it fast to its appropriate thread upon the pyramidal bed-plate; *the advantage being that the other parts are common to all.*

One great advantage with such an instrument is, that where large numbers of sections are required for purposes of instruction, in the working laboratories of our medical schools, there can be a great saving of time, both of student and instructor, and also of material; and, moreover, it especially facilitates the matter of class demonstrations, for all the members can be looking at practically the same locality of a growth at the same time. *Large and very thin sections can readily be obtained, and at the rate of eighty a minute, or more than one each second.* The delicacy, ease and rapidity with which they can be cut must be seen in order to be appreciated.

The art of cutting is very readily acquired, and when the preparation is frozen, it is but the work of a very few moments to obtain several hundred sections. It is not necessary to remove the sections from the knife every time, but twenty or thirty may be permitted to collect upon the blade. They lie curled or folded up upon the knife, and when placed in water straighten themselves out perfectly in the course of a few hours. These sections are not only evenly cut, but show no traces of irregularity from the knife. They consist of very nearly a single layer of elements, and form exquisite objects for mounting, more especially for examination with high powers. Several hundred sections are made from a single specimen, (histological or pathological), and kept in a preservative fluid, recommended by Dr. David J. Hamilton. It is composed as follows: ℞. Glycerinæ, Aquæ distill., aa., ℥iv. Acid Carbolici, gtts., iij. Boil and filter.

Sections keep indefinitely in this preservative,* and

*I have specimens which have kept perfectly in this solution for about a year.

whenever desired a section may be selected almost at random, and mounted. I have obtained many fine preparations by this method, and use it constantly in the laboratory, for the reason that a small amount of material can be made to go so far. For example: one can make two or three hundred sections from a single epithelioma of small size, or perhaps utilize the whole of some rare pathological growth by converting it all into sections, almost every one of which is capable of exhibiting satisfactorily the characteristics of the new formation. Thus one is enabled to simplify his work in each department, and not as hitherto be considered entirely dependent upon the opportunities of the moment for supply in matters of pathological interest.

After a careful trial of Hamilton's method of section cutting, it occurred to me that it might be possible to cut perfectly fresh tissues without special preparation, other than by simply freezing them. I attempted to make transverse sections of two adult kidneys, from the same individual, a contracted kidney, seven-eighths of an inch diameter, and a large white kidney, one and seven-eighths inches diameter, and succeeded beyond my expectations. I obtained entire sections of both kidneys, from capsule to pelvis, and sufficiently thin for immediate microscopic examination. By using osmic acid a satisfactory demonstration of the pathology of Bright's disease was obtained; for, by means of the reaction of the acid, those tubules containing fatty degenerated epithelium were marked out with great distinctness, and one could clearly trace through the medullary portion, and along the pyramids, the course of many of the converging collecting tubules in which the process was more advanced than in others, and in which masses of fatty degenerated epithelium were blocking up the interior. The picture that these

long collecting tubules presented, with their fatty contents, was quite striking. This method could be employed to very good advantage for purposes of instruction.

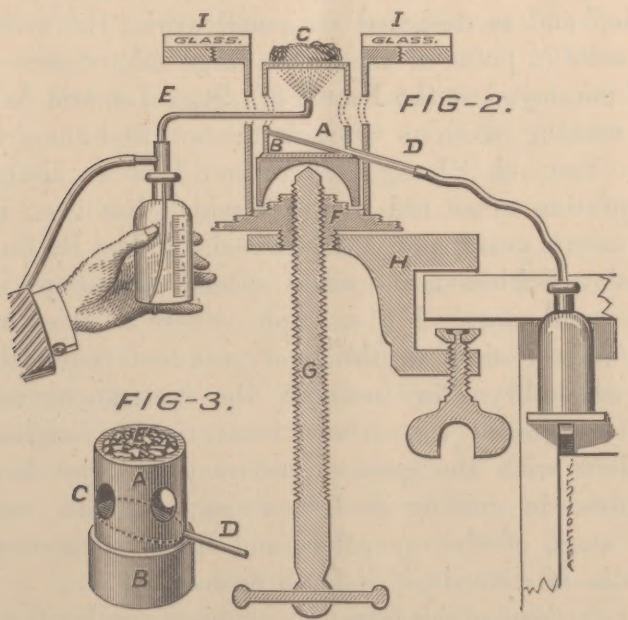
The freezing by ice and salt requires at least twenty minutes of patient, "stirring" work, and when several specimens are to be cut the outlay of considerable time. It was, therefore, with great satisfaction that I read an able and thoughtful article by Bevan Lewis, F. R. M. S., etc., in the October number of *Brain*, entitled "Application of Freezing Methods to the Microscopic Examination of the Brain," in which he recommends the use of ether spray, and employs a hollow cylinder of zinc, with a false sloping bottom leading to an exit tube, which conducts off the condensed ether. This cylinder "possesses three circular openings, three-quarters of an inch in diameter; one placed in front of the two others laterally opposite each other." "The nozzle of the spray instrument is introduced through the opening in the left hand side of the freezing chamber, and the spray made to play upon the lower surface of the cap immediately beneath the tissue to be frozen." It occurred to me that, instead of using the ice and salt in the microtome just described, the ether spray could be employed with some modifications; and so, having a zinc cylinder made a little smaller than one of the wells of the poly-micro tome, I found that freezing required only about a minute, and consumed about six drachms of ether, a portion of which was re-collected.

I was pleased to find such good results from so simple a contrivance, and to observe how easily and quickly thin layers of tissue could be frozen, and how readily a portion of the ether could be re-collected without any elaborate condensing apparatus; for "the free evaporation and subsequent condensation of the ether is provided for

in the same chamber." Of course, in such an imperfect arrangement a portion of the ether is lost. Anyone having a microtome can convert it into an ether freezing microtome, by simply having a plain zinc cylinder, such as has been described, made a little smaller than the well of his microtome, "soldering it fast to the plunger to insure absolute steadiness," and then with the ordinary spray instrument freeze the specimen; drop it quickly into its place, and cut in the usual way. If a more perfect instrument is desired, it is only necessary to have holes cut through the wall of the microtome to correspond with the openings in the zinc cylinder, also a narrow slot for the exit tube for conducting off the condensed ether, and everything for freezing is complete—*voilà tout.* The following drawing will perhaps assist in the explanation:

POLY–MICROTOME.

ARRANGED FOR FREEZING WITH ETHER SPRAY.



EXPLANATION OF FIG. 2.

(Drawing—One-fourth the original size).

A—Zinc cylinder or spray chamber. B—False or sloping bottom for conducting of condensed ether. D—Exit tube leading to collecting bottle. C—Object to be frozen. E—Ether spray apparatus. F. Pyramidal bed-plate, etc., etc.

EXPLANATION OF FIG. 3.

A—Zinc cylinder or spray chamber. B—Plunger of microtome. C—Opening for spray instrument, etc. D—Exit tube for collecting condensed ether. E—Roughened top to facilitate the retention of the frozen object in position.

The instruments just described are best employed for certain kinds of work only, and would prove inadequate if called upon to perform other work than that for which they had been especially constructed; consequently, other instruments have been devised.

It may be well to make mention of some other varieties in this connection. I would therefore refer to two other forms. One is known as the "Long microtome," and is designed for small work; the other, its opposite in point of size,—the large microtome—made and employed at the New York State Lunatic Asylum, for making sections through the entire human brain. The first, or "Long's microtome," is an improved adaptation of an old principle which has been in use for many years, and was devised by Dr. R. Long, of Breslau, Schlesien, and offers special advantages in certain particulars. For example, where a series of successive sections of anything very minute is required, such as, an embryo for instance, the instrument can be easily adjusted for either transverse or longitudinal sections with the greatest accuracy, and no haste is required in cutting such a series; one can cease at any stage of the operation, and begin again days or months afterwards, if it is so desired.

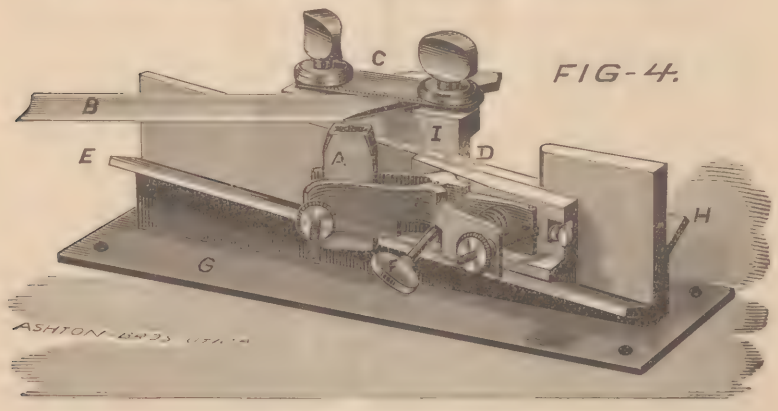
In speaking of this form of instrument another fact must also be mentioned in connection with it. I refer to a new

embedding mass which was first brought to the notice of the profession by the late Prof. Calbarla, of Heidelburgh, and who died a short time since in the south of France. The mass bears his name, and is known as the "Calbarla mass," and consists simply of eggs and a ten per cent solution of carbonate of soda. It is prepared as follows: White of egg, fifteen parts; solution carbonate of soda, (ten per cent,) one part; mix well, and add the yolks of the eggs from which the albumen was obtained. The whole should be well mixed together, and all fragments of vitelline membrane and *chalaze* should be carefully removed. This is called fluid Calbarla. The object to be embedded is fastened upon a piece of old Calbarla, and placed in a paper box, and the new fluid, Calbarla mass, is poured upon it, and the whole placed in a small *covered* porcelain vessel, with alcohol surrounding it, and this placed upon a water bath and heated to about 50° C. It is then allowed to cool. The mass will be found coagulated into a substance resembling custard, and all that remains to do is to remove the paper box surrounding the specimen, and drop it into alcohol. The mass gradually attains the proper degree of hardness by the alcohol abstracting the water from it. It is one of the most satisfactory masses to cut, when properly prepared, that one could desire, and supplies a need which has long been felt by those working in exceedingly delicate and fragile objects. This mass is permeable to spirits, and consequently objects can remain embedded in it for any length of time without injury. It is also very useful where there are delicate formations or irregular jutting processes, joined by slender membranous connections, for it holds and preserves them in all their important relations, oft-times absolutely necessary, as, for example, the preservation

of relations of the epiblast, mesoblast and hypoblast.

It also permits the subsequent arrangement of the sections upon the slide. I have placed as many as thirty-two separate sections, through cerebral hemispheres, optic and otic vesicles, in perfect order, under an ordinary sized cover glass.

The following is a cut of the "Long microtome:"



EXPLANATION OF FIG. 4.

(Drawing—One-third the natural size).

A—Object embedded in "Calbarla." B—Knife. C—Knife-clamp. H—Horizontal slide for carrying knife. E—Inclined slide, (angle 5°), for carrying object. F—Set-screw to return clamp at any angle. D—Vernier scale. G—Bed-plate.

The object is kept moist with alcohol from a dropping-flask, or from an ordinary wash bottle filled with alcohol.*

It possesses a Vernier scale, and fine readings can be obtained. Sections one-five hundredth of an inch or even thinner can be cut, and one need not necessarily lose one section in fifty, so perfectly does this instrument work, especially when used in connection with the "Calbarla mass."

A word concerning very large sections. It happened to be my good fortune to have been at Munich,

* This instrument is constructed of brass, and costs with two knives, in Breslau, 60 marks, or about $15.

in August last, and to have visited the "Kreis Irren Anstalt," under the directorship of Prof. Gudden. I received a most cordial welcome, and spent some days in the laboratory there with Dr. Forel, first assistant, to whose faithful and patient work the results obtained are due, almost as much as to the brilliant and successful experiments made by Prof. Gudden himself. These sections were very evenly and nicely cut, but they were not intended for examination with high powers, and were simply covered by what appeared to be crown glass, and were used to make drawings from, being enlarged by means of a hand glass or loupe In fact, I did not perceive any microscope with a stage sufficiently ample to accommodate so large a section as that through the entire human brain.

The special pathologist, of the State Lunatic Asylum at Utica, Theodore Deecke, has recently been engaged in making vertical sections through the entire human brain, and has now reached a point midway between the anterior and middle commissures. He has thus far, (in this single brain), made one thousand sections, and the total number for the entire brain will probably aggregate two thousand. Each section is one-four hundredth of an inch in thickness, but when desired a section of one one-five hundredth of an inch can be obtained. They are very perfect, and are mounted in such a manner as to be available to one-fifth inch or one-eighth inch objective in any part of their area.*

The study of the anatomy of the brain possesses positively a new and fascinating attraction when pursued by this novel method; for what can be more conducive to study than each day to witness different points of interest appearing, as the brain is slowly cut away.

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*For a full description of microscope and microtome, see AMERICAN JOURNAL OF INSANITY, July, 1876.

The advance is so very gradual that the various important parts become indelibly stamped upon the memory; for this method gives as it were a complete typographical survey of the brain. The various convolutions of the brain mantel, with pia and blood vessels; the different fissures and sulci appearing and disappearing; the Island of Reil, and its relations to the cortex; the great ganglionic centers, ependyma and ventricles; or the location and distribution of the various commissures, all stand out as familiar landmarks to guide one in his examinations in subsequent pathological investigations.

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The JOURNAL is now at the close of its thirty-fifth volume. It was established by the late Dr. Brigham, the first Superintendent of the New York State Lunatic Asylum, and after his death edited by Dr. T. Romeyn Beck, author of "Beck's Medical Jurisprudence;" and since 1854, by Dr. John P. Gray, and the Medical Staff of the Asylum. It is the oldest journal devoted especially to Insanity, its Treatment, Jurisprudence, &c., and is particularly valuable to the medical and legal professions, and to all interested in the subject of Insanity and Psychological Science